Assembly of all Syn construct

Prepare Gibson reaction

1.2

	PCR product pNF dilute 5X	HO glnA Up (pNF)	2 KmR casette	1,2 pTrc	2 AckA	2 HO glnA Dw (pNF) dilute 2X	Mastermix
Conc (ng/µL)	140.8	29,1	64,6	44,9	15,3	113,1	---
Volume (µL)	1	2	1	1	4	1	10

1.4

	PCR product pNF dilute 5X	1.3 DO acs UP	3,4 SpR casette	4 pTrc	4 pta	4 HO acs DW (pNF)	Mastermix
Conc (ng/µL)	140.8	6.1	35.9	45.7	34.9	91.9	---
Volume (µL)	0	0	0	0	0	0	10

1.6

	PCR product pNF dilute 2X	HO glnA Up (pNF)	1,2,5,6 Kmr casette	6 HO glnA Dw (DO)	Mastermix
Conc (ng/µL)	64.9	24.9	37	63.4	---
Volume (µL)	1	4	3	2	10

1.7

	PCR product pNF dilute 2X	3,4,7 HO acs Up	3,4,7 SpR casette Dilute 2X	7 acs Dw (DO)	Mastermix
Conc (ng/µL)	64.9	5.2	56	51.8	---
Volume (µL)	1	7	1	1	10

Gibson reaction

50 degrees 2 hours

Transform to *E. coli* with standard heat shock protocol (3 tubes of ecoli)

- Transforms 5 µL and 2 µL of Gibson assembly to E. coli
- Do a negative control: transform 5 µL of 40X diluted PCR pNF (40X dilution: 1 µL of cut pNF + 39 µL sterile MQ water). This will transform 8.1 ng of PCR product.
- Plate both on LB + amp
- 37 degrees' overnight

Replate

- Restreak 1.2 colonies + incubate in LB + amp overnight 37 degrees
- Miniprep

Restriction verify with BcuI and PaeI

	Plasmid
Sterile MQ water	Up to 10 µL
10X FastDigest **Green** Buffer	1 µL
Construct 1.2 (miniprepped)	150 ng (if higher conc than 150 ng/µL, use 1 µL)
BcuI	0.5 µL
PaeI	0.5 µL
Total	10 µL

60 min 37 degrees

Load 10 µL (or 150 ng if the restriction contains more than 150ng) on gel. Post stain with GelRed. Should look like **lane 1**

MW: GeneRuler™ 1 kb DNA Ladder

1: Assembled glnA KO + AckA insert

BcuI + PaeI

1. 5502 bp

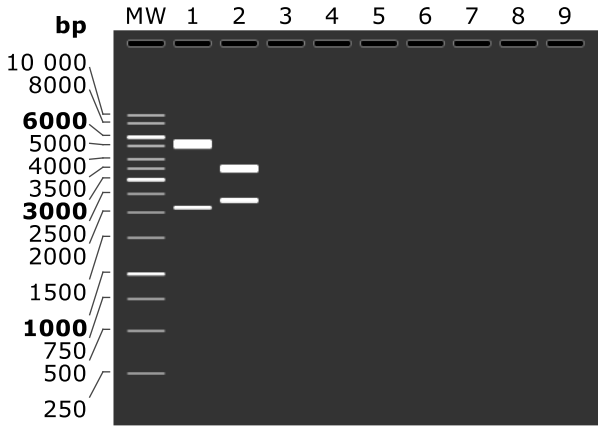
2. 2139 bp

2: pNF_Delta-phaA_KmR -**empty plasmid**

BcuI + PaeI

1. 3668 bp

2. 2329 bp



Send to sequencing if correct. Gogo syn transformation!!